THE RESISTANCE OF EMBRYONIC EPITHELIUM, TRANSPLANTABLE MOUSE CANCER, AND CERTAIN ORGANISMS TO FREEZING WITH LIQUID AIR.*

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THERE appeared recently in the *Lancet*[1] an article by J. E. Salvin-Moore and C. E. Walker on the relationship of cancer cells to the development of cancer, and also a note by J. E. Salvin-Moore and J. O. Wakelin Barratt on the effect of liquid air upon the graftable cancer of mice. Both of these articles deal with experiments made by Moore, Walker, and Barratt in the action of liquid air upon transplantable cancer in mice. The facts and conclusions of each article are practically the same, the first dealing with a transplantable mouse cancer received from Ehrlich, the latter with the Jensen mouse cancer. These authors find that transplantable mouse cancer can be exposed to the freezing of liquid air at $-195°$ C. for from 20 minutes to half an hour and that such frozen material inoculated into mice is capable in a certain number of instances of producing growing tumors. It is obvious from these articles that the tumors which develop from such grafts are of essentially the same histological appearance as the tumors from which they were taken. Although these authors give prominence to the idea that the freezing in all probability destroys the cancer cells, but leaves intact some virus that stimulates the cells of the host to proliferation with the formation of a new tumor, they consider the possibility of the cancer cells being able to withstand this low temperature. Inasmuch as some bacteria and trypanosomes are said to survive this temperature for a period of 20 minutes, and as normal tissue cells are not supposed to be capable of resisting such temperature, they are inclined to believe that these experiments indicate the presence of a parasite in the cancer tissue.

For the purpose of repeating Moore, Walker, and Barratt's experiments, and for the purpose of control, the following experiments were

* Received for publication August 19, 1908.

[1] *Lancet*, January 1908, 174, p. 226.

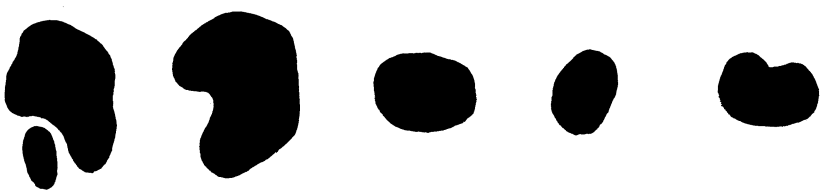
undertaken: A rapidly growing carcinoma of the mouse, which was giving a high percentage of inoculations, known as G 1,532 D was divided into three portions. The first portion was frozen 40 minutes, then injected into 9 susceptible white mice known as Lot 1,561. The second portion was frozen for one hour and 20 minutes, and injected into 14 white mice known as Lot 1,562; and the third portion, without freezing, as control, was injected into five susceptible mice, known as Lot 1,563. The following table shows plainly the procedure followed.

TABLE 1.

LOT 1,532 D. MARCH 27, 1908.

Lot 1,561, 9 Mice, Tumor Frozen 40 Min.	Lot 1,562, 14 Mice, Tumor Frozen 1 Hr. 20 Min.	Lot 1,563, 5 Mice, Control	Killed
A, nil.	A, nil.	A, beg. tumor	March 31, 1908
B, nil.	B, nil.	B, " "	April 1, 1908
C, nil.	C, nil.	C, " "	April 3, 1908
D, nil.	D, nil.		April 6, 1908
E, nil.	E, nil.		April 9, 1908
F, beg. tumor	F, nil.		April 14, 1908
G, large tumor	G, died April 28, 1908		June 15, 1908
H, nil.	H, died May 20, 1908	} Large tumors	
	K, still living		
	4 nil.	E	April 20, 1908 } large
		F	April 20, 1908 } tumors

All of the controls developed tumors. A, B, and C were killed on the fourth, fifth, and eighth days respectively. E and F were permitted to grow and on the twentieth day had reached the size shown in the accompanying diagram, when the animals were killed. Among the tumors frozen 1,561 G developed a tumor which was allowed to grow until of large size (see diagram). It was killed on



1563 E. 22 days. | F 22 days. | 1561 G. 13 days. | 1562 G. 30 days. | 1562 H. 52 days.

the thirteenth day. No. 1,562 G developed a more slowly growing tumor, the animal being killed on the thirtieth day (see diagram). No. 1,562 H died on the fifty-second day with a tumor of considerable size (see diagram).

Of the tumors removed in the earlier stages of the process, 1,561 F showed growing tumor cells. In 1,561, of nine mice inoculated with tumor frozen 40 minutes, two tumors developed. In Lot 1,562, 14 mice, material frozen 1 hour and 20 minutes, three developed tumors which were permitted to grow until of considerable size. The controls, five mice, Lot 1,563, gave 100 per cent of rapidly growing tumors.

For the purpose of determining the resistance of trypanosomes to freezing with liquid air, we employed the spleen of rats infected with *Trypanosoma gambiense.*[1] This organism has a degree of virulence which kills normal animals in three days. The infected spleens were taken on the last day of the disease and divided into four portions, one of which was frozen for 20 minutes and injected into two rats, the second portion was frozen 40 minutes and injected into two rats, the third portion was frozen 1 hour and 20 minutes and injected into two rats, and the fourth portion injected direct into two rats as control. The following table shows results.

TABLE 2.

TRYPANOSOMA MATERIAL FROZEN. RAT A-1. MARCH 31, 1908.

Examination of Blood	Lot 343, 2 Rats, Mat. Frozen 20 Min.	Lot 344, 2 Rats, Mat. Frozen 40 Min.	Lot 345, 2 Rats, Mat. Frozen 1 Hr. 20 Min.	Lot 346, 2 Rats. Control
April 3, 1908.....	1–2 Tryps.	0	0	Died April 3, 1908
April 6, 1908.....	Died	0	0	
April 9, 1908.....		0	0	
		Still living	Still living	

From this it will be seen that *Trypanosoma gambiense* can withstand freezing with liquid air for a period of 20 minutes, although its virulence is somewhat injured, the animals inoculated dying on the sixth and ninth days respectively after inoculation, whereas both the controls died on the third day. Animals inoculated with the organism after freezing 40 and 80 minutes respectively remained uninfected, the organism evidently being killed by exposure to freezing for these periods.

For the purpose of determining what is the resisting power of growing epithelium to freezing, we employed the tissues of young mice embryos, removed aseptically, and after freezing 20, 40, and

[1] The infected rat from which these animals were inoculated was kindly given us by Dr. J. L. Todd.

80 minutes respectively, the materials were injected into mice with suitable direct controls, as shown in the appended Table 3.

TABLE 3.

EMBRYONIC MOUSE MATERIAL FROZEN IN CONTROL FOR TUMOR 1,532 D. MARCH 27, 1908.

Lot 1,564, 9 Mice, Frozen 20 Min.	Lot 1,565, 8 Mice, Frozen 40 Min.	Lot 1,566, 14 Mice, Frozen 1 Hr. 20 Min.	Lot 1,567, 5 Mice, Control	Killed
A	A	A	A	March 30, 1908
B	B	B	B	April 1, 1908
C	C	C	C	April 3, 1908
D	D	D		April 6, 1908
E	E	E		April 9, 1908
F	F	F		April 14, 1908
1 left	1 left	2 left	all dead	June 3, 1908

For purposes of determining the evidences of growth animals from each group were killed on the fourth, fifth, seventh, tenth, and twelfth days, the implanted embryonic tissue hardened and embedded and subjected to microscopic examination. In each case tumor material, exposed to the same length of freezing and removed on the same day, was at our disposal for comparison. In the case of embryonic tissue it is well known that epithelium transplanted into the subcutaneous tissue of animals of the same species shows distinct evidence of proliferation and growth frequently for a considerable period of time. Such evidences of proliferation can be found in the cells of the implanted embryonic tissue as early as 24 hours after implantation and frequently continue for weeks and even months, producing tumor-like growths which ultimately retrograde and are finally absorbed and removed. To a less extent a similar phenomenon has been observed in transplanted adult epidermis. The epidermis

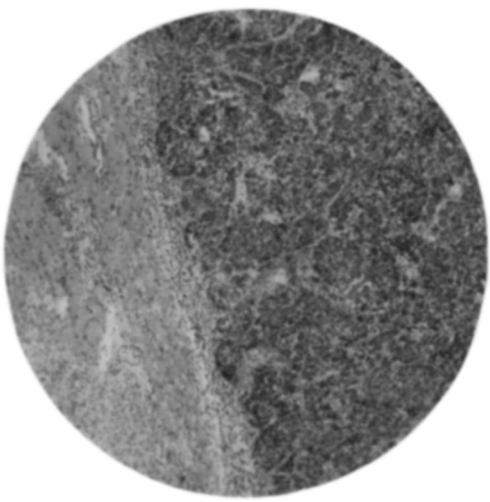


FIG. 1.—Section of mouse tumor, 1,562 G, killed 30 days after inoculation. Shows margin of rapidly-growing, soft, solid carcinoma of the breast, presenting the histological characteristics of the tumor from which the material for inoculation was taken. Material frozen 80 minutes with liquid air.

of guinea-pigs implanted subcutaneously in the guinea-pig, where it is able to form cystlike structures, persists for a considerable period, but is ultimately absorbed. In all the controls of our experiments with embryonic tissue, the embryonic tissue showed evidences of growth. In three of them which were removed on respectively the fourth, fifth, and seventh days, evidences of proliferation of epithelium were distinct. Fig. 2, microphotograph from control removed on the fifth day, shows the characteristic formations of epithelial cysts and proliferation of the epithelium into the surrounding connective tissue. All of the embryonic tissue exposed to freezing with liquid air failed to grow and the implanted fragments removed at various periods show complete necrosis of the embryonic tissue and in the later specimens growth of connective tissue and evidences of phagocytosis and advancing absorption. Fig. 3 from a specimen removed on the fifth day after freezing 20 minutes

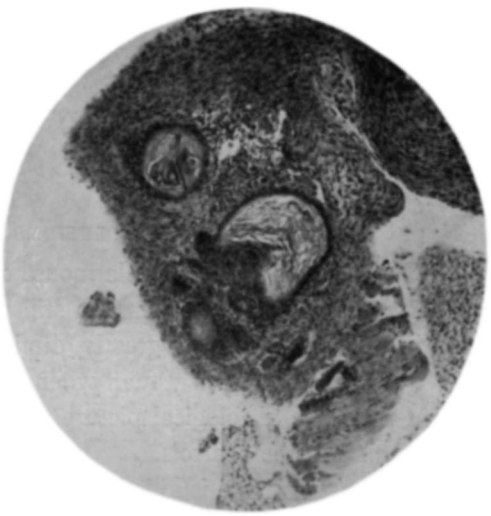


FIG. 2.—Growing embryonic epithelium in the subcutaneous tissue of mouse. Characteristic cystlike formations in the epidermis. Proliferating epithelium from the stratum granulosum.

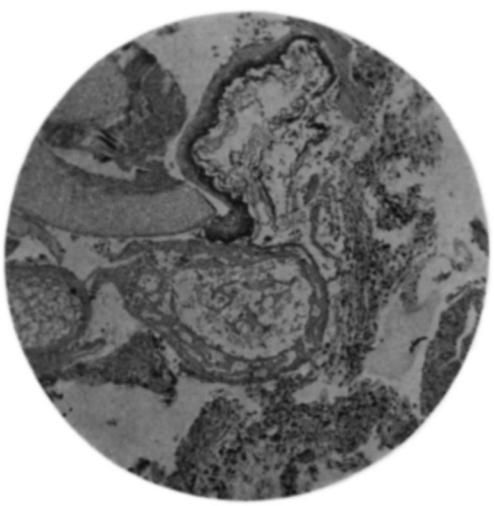


FIG. 3.—Embryonic tissue after freezing 20 minutes. At the margin connective tissue of the host. Remnants of intestinal epithelium, cutaneous epithelium cartilage and muscle, all in an advanced stage of necrosis.

with liquid air well illustrates the total necrosis of the tissue.

From these experiments it will be seen that embryonic tissue is incapable of withstanding the low temperature developed by freezing with liquid air; that established transplantable cancer of the mouse is capable of withstanding a period of freezing as long as 80 minutes; that *Trypanosoma gambiense* can withstand freezing for 20 minutes, but not for 40 minutes.

CONCLUSIONS

1. The cells of transplantable mouse cancer can withstand freezing for a period of 80 minutes and still produce tumors. The percentage of inoculations is greatly diminished, the tumors appear later and grow more slowly than when transplanted directly. They present the same histological picture as the tumors from which they were taken and the controls.

2. Embryonic tissue is killed by freezing with liquid air.

3. *Trypanosoma gambiense* can resist freezing with liquid air for a period of 20 minutes. It is killed at 40 minutes.